Osteoarthritis and Cartilage 18 (2010) 810–816

Osteoarthritis and Cartilage

Interleukin-4/interleukin-4 receptor gene polymorphisms in hand osteoarthritis

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ARTICLE INFO

Article history:
Received 18 June 2009
Accepted 9 February 2010

Keywords:
Hand Osteoarthritis
Gene Polymorphism
Cytokines
IL-4 Receptor
Association Study

SUMMARY

Objective: IL-13/IL-4/IL-4R system has strong chondroprotective activity. We investigated polymorphisms in these genes as potential hand osteoarthritis (OA) susceptibility loci by performing a case–control association study.
Methods: Eighteen common single nucleotide polymorphisms (SNPs) (nine in *IL-4R*, five in *IL-4* and four in *IL-13*) were genotyped in 403 patients (380 females) with hand OA and 322 healthy controls (308 females).
Results: Two SNPs (rs1805013 and rs1805015), mapping to the *IL-4R* gene, were associated with *P*-values of 0.0116 and 0.0305 respectively in the whole sample. As far as the non-erosive hand OA group ($n = 159$) is concerned, the significance level of association of SNP rs1805013 is increased. After correction for multiple testing (correction for the 54 tests) the significance was not retained.
None of the *IL-13* SNPs analyzed showed association with hand OA. Some of the analyzed SNP within the *IL-4* gene showed significant association with hand OA only when considering subgroups of patients. With respect to the CMC1 OA group, two SNPs in *IL-4* (rs2243250 and rs2243274) showed association with a *P*-value of 0.027 and 0.018 respectively. None of these associations remained after correction for multiple testing.
Conclusions: The present study shows a trend to an association between non-erosive hand OA in Caucasian population and a genetic variant in the coding region of *IL-4R* gene. Our results, in keeping with previous data on hip OA, confirm the suggestion that *IL-4*/*IL-4R* system plays a role in OA pathogenesis. Further confirmation studies on different populations are necessary.

Introduction

Osteoarthritis (OA) is a condition for which genetic predisposition has been intensively studied[1–3]. In the past 10 years a large number of twin pairs, familial recurrence, segregation studies and association studies have suggested the presence of major genetic components underlying a complex mode of inheritance[4–11].

OA can affect different skeletal sites such as knee, hip, hand and spine. All genetic studies hitherto conducted have generated different results for different sites, which suggest site-specific genetic influence for OA[10]. As for most complex diseases, both inherited genetic variations and environmental influences combine to influence the disease risk. Among the recognized environmental factors for OA are gender, obesity, joint instability, repetitive injury (microtrauma) with overloading of joints, and loss of muscle mass and strength. Nevertheless many people exposed to adverse lifestyle factors do not develop the disease, suggesting that there are susceptibility and resistance factors which mediate the risk of environmental influences, such as chondrocyte and bone cell response to various mechanical and molecular stimuli[12]. All these phenomena are genetically determined and involve the precise regulation of enzyme and cytokine production which are the major responsible of the imbalance between chondrocyte anabolic and catabolic activities[13].

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doi:10.1016/j.joca.2010.02.005

Chondrocytes from OA cartilage show impaired response to mechanical stress mediated by the interleukin-4/interleukin-4 receptor (IL-4/IL-4R) system. Cartilage chondrocytes function and integrity are regulated by mechanical stimulation and IL-4 is a major active autocrine/paracrine signalling molecule in this mechanotransduction pathway[14,15]. In order to initiate signalling, IL-4 binds to the IL-4Rα chain, which then forms heterodimers either with the common γ chain to form type 1 receptor or with the IL-13Rα1 subunit to form type 2 receptor. It has been proposed that the latter form of IL-4R can be activated by both IL-4 and IL-13[16]. Formation of the different receptor types results in the activation of alternative downstream signalling pathways. IL-4 and IL-13 are strong chondroprotective cytokines and it is reasonable to speculate that polymorphisms within their genes or their receptor genes may be risk factors for OA.

Previous studies have suggested that the allelic variations of the genes of IL-4/IL-4R system are related to chondrocyte response to mechanical stimulation. Different single nucleotide polymorphisms (SNPs) in *IL-4R* gene have an assessed functional role in regulating signal transduction downstream this receptor. In particular rs1805015 (S503P) and rs1801275 (Q576R) can affect the binding and phosphorylation of the intracellular substrates STAT6 and IRS[17]. Soluble IL-4R protein levels is regulated by a variant (rs2057768, −589C/T) mapping in the 5′ promoter region of this gene[18]. In addition, among the different susceptibility loci for OA identified so far through linkage analysis, a susceptibility locus for hip OA maps on chromosome 16p2.3–p12.1 in a region containing the IL-4Rα chain gene[6,19]. Four different non-synonymous coding SNPs of *IL-4R* (S436L, S503P, Q576R, S752A) from this region were found associated with an increased risk for hip OA[19].

We therefore investigated polymorphisms in these genes as potential hand OA susceptibility loci by performing a case–control association study with 18 common SNPs: five in *IL-4* (NM_000589), nine in *IL-4R* (NM_000418) on chromosome 16 and four in *IL-13* (NM_002188) on chromosome 5.

Patients and methods

Patients

A total of 403 Caucasian patients (23 males and 380 females, age range 41–84 years, mean 64 years) were recruited by three Rheumatological Centres in Italy (Bologna, Padova, Siena). In addition 322 Caucasian controls (14 males, 308 females, age range 42–84 years, mean 62 years) were selected among unrelated individuals showing no clinical signs of osteoarticular disorders who attended orthopaedic or rheumatologic outpatient clinics of the Rizzoli Orthopaedic Institute (Bologna) for minor non-specific complaints.

From all patients past and present medical history was taken and detailed musculoskeletal examination was performed, in order to exclude patients with inflammatory arthritides and/or with psoriasis or with positive family history of psoriasis. Clinical OA in other sites (hip[20], knee[21]) was also recorded. Radiographs of hip and knee were obtained when clinical involvement was detected. Plain hand radiographs were obtained from all patients. Hand OA diagnosis was made according to the American College of Rheumatology Clinical Criteria for Hand OA[22].

All radiographs were scored for joint damage following the Kellgren–Lawrence and the Kallman scores[21,22]: two rheumatologists, with experience in hand radiograph scoring, assessed X-ray films together at the same time; the readings were jointwise; the sums of single joint scores from each patients were obtained and used in statistical analysis. When necessary, consensus was reached by discussion and agreement. The second reading was carried out on the first 30 consecutive X-rays and intraclass coefficient values for intrareader reliability for Kellgren–Lawrence scores at a single joint was 0.985 (0.983–0.988) and for Kallman was 0.988 (0.986–0.990). In addition to Kellgren–Lawrence and Kallman scores, we also utilized the scores relating to osteophytes and to joint space narrowing (items of Kallman score). X-ray readers were unaware of the genetic results.

Patients were then divided into erosive and non-erosive hand OA subsets according to the presence or absence of well defined typical erosive changes (gull-wing and saw-tooth appearance) in two or more digits. In this way we identified 197 patients with the erosive form (14 males, 183 females, age range 44–83 years, mean 64 years). In the non-erosive form we included 159 patients with no sign of erosion anywhere (9 males, 150 females, age range 43–84 years, mean 62 years). Therefore 47 patients were left after this selection because they showed an intermediate form (either erosion in one digit, or less typical or initial form of gull-wing/saw-tooth pattern). First carpometacarpal joint (CMC1) OA was present in 166 patients. Finally among the total patient population, 59 cases had concurrent hip OA and 95 concurrent knee OA.

Venous blood was drawn from each patient/control between 9.00 and 12.00 AM and immediately processed for DNA extraction.

Ethical approval for the study was obtained from appropriate ethics committees and informed written consent was obtained from all subjects.

Genotyping

DNA was extracted from white blood cells from plasma samples by means of the DNA Isolation Kit for Mammalian Blood (Roche, Indianapolis, IN). DNA was quantified with PicoGreen dsDNA Quantization Kit (Molecular Probes, Eugene, OR).

Genotyping was performed using TaqMan® SNP Genotyping Assays (Applied Biosystems). For each marker 20 ng of DNA was amplified in a 10 μl reaction volume on a Applied Biosystems 7500 Fast Real-Time PCR System. TaqMan® Pre-Designed Assays had been used for 15 SNPs and Custom TaqMan® SNP Genotyping Assays had been used for the remaining three SNPs. Additional SNP details are provided in Table I. The cycling parameters used for TaqMan reactions is as follows: 95°C for 10 min; 40 cycles of 95°C for 15 s followed by 60°C for 1 min.

Statistical analysis

The Kruskal–Wallis ANOVA analysis (for unpaired and non-parametric data) and Dunn's multiple comparison correction test was applied to compare radiological scores among OA patients subgroups classified according to major and minor allele carriage of *IL-4R* SNP rs1805013. Analysis was performed using GraphPad Prism for Windows (CA, USA).

Genotype and allele frequencies were compared between OA cases and controls using Plink software (http://pngu.mgh.harvard.edu/purcell/plink/)[23], and odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated. Correction for multiple testing was performed with Plink which generate the adjusted significance values by using Bonferroni, Holm and Sidak correction methods. Independent effect of associated SNPs has been evaluated using Plink. Hardy–Weinberg (HW) equilibrium, and haplotype frequencies were estimated from the genotype data using Haploview software, which uses the expectation-maximization (EM) algorithm[24]. Pair-wise linkage disequilibrium (LD) in our sample between the individual SNPs was calculated using the LD-plot function of this software. Comparisons of the distributions of allele, genotype and haplotype frequencies were performed using the chi-square test. SNPs with a call rate <95% or departure from HW

Table I
Data quality table for the input file. For each SNP it is indicated the TaqMan® SNP Genotyping Assays assay ID when a Pre-Designed SNP Assays has been used (assay ID), the alleles (for each SNP the two allele are indicated as major allele: minor allele), the gene, the NCBI SNP database ID (dbSNP ID), the location within the gene with the amino acid substitution when a coding SNP has been studied (location), HW equilibrium *P*-value in the control group (HW *P*-value) and the minor allele frequency (MAF)

Assay ID	Alleles	Gene	dbSNP ID	Location	HW *P*-value	Call rate	MAF
C_11740467_10	A:C	IL-13	rs1881457	Promoter (−1512)	0.2667	98.8	0.194
C_8932056_10	C:T	IL-13	rs1800925	Promoter (−1112)	1	99.2	0.196
Custom Assays	G:A	IL-13	rs20541	Exon 2 (R144Q)	0.8326	95.7	0.186
Custom Assays	G:A	IL-13		Exon (+4738)	0.632	99.2	0.187
C_16176216_10	C:T	IL-4	rs2243250	Promoter (−589)	0.5025	99.9	0.121
C_16176215_10	C:T	IL-4	rs2070874	5'UTR(−33)	0.3079	98.6	0.116
C_11818513_1_	G:T	IL-4	rs2227284	Intron 2 (+3353)	0.0903	97	0.229
C_15751521_10	G:A	IL-4	rs2243266	Intron 2 (+4417)	0.5027	98.6	0.111
C_16176468_10	G:A	IL-4	rs2243274	Intron 2 (+5460)	0.3294	99.2	0.122
C_2769607_10	C:T	IL-4R	rs2057768	Promoter (−3223)	0.321	97.7	0.265
Custom Assays	C:T	IL-4R	rs2107356	Promoter (−1914)	0.153	96.7	0.43
C_2769554_10	A:G	IL-4R	rs1805010	Exon 6 (I75V)	1	99.6	0.443
C_2769552_10	C:T	IL-4R	rs3024571	Exon 7 (N167N)	0.6762	99.7	0.109
C_8903098_20	A:C	IL-4R	rs1805011	Exon12 (E400A)	0.8524	99	0.082
C_8903093_10	C:T	IL-4R	rs1805013	Exon12 (S436L)	0.317	99.4	0.067
C_8903092_20	T:C	IL-4R	rs1805015	Exon12 (S503P)	0.3763	99	0.177
C_2351160_20	A:G	IL-4R	rs1801275	Exon12 (Q576R)	0.9775	99.9	0.186
C_8903091_10	T:G	IL-4R	rs1805016	Exon12 (S752A)	1	98.5	0.074

equilibrium in the control group (exact test $P < 0.01$) were excluded from the final analysis.

Results

All 18 SNPs conformed to the HW equilibrium in the control group ($P > 0.05$) (Table I).

The case–control association analysis was performed first on the whole sample regardless of the gender and the clinical features, and only two SNPs (rs1805013 and rs1805015), mapping to the *IL-4R* gene on chromosome 16, were associated with *P*-values of 0.0116 and 0.0305 respectively (Tables II and III). This association was attributable to an increased occurrence in the affected individuals of the minor allele, the T and C alleles respectively, at these SNPs. The *IL-4R* SNP rs1805013 has been already reported associated with an increased risk of hip OA[19]. As some of our patients developed also hip OA, we wondered whether our association results were due to this small subgroup of patients. However, the association with this SNP was maintained also when subtracting from the whole sample the patients who developed hip OA (*P*-value = 0.017) (Tables II and III). When considering only patients not developing knee OA, the association remains still significant for this SNP (data not shown).

According to the presence or absence of well defined typical erosive changes in two or more digits, patients were divided into erosive and non-erosive hand OA subsets. As far as the non-erosive hand OA group ($n = 159$) is concerned, the significance level of association of SNP rs1805013 showed an increase with a *P*-value of 0.002 and it showed a nominally significant *P*-value after correction for multiple testing (*P*-value = 0.018; OR = 2.206 [95% CI = 1.32–3.68] after correction for nine tests [number of markers tested for the *IL-4R* gene]; non-significant after correction for 54 tests [total number of multiple tests done]). In addition, also the other SNP of *IL-4R* gene, rs1805015, showed an increased association with a *P*-value of 0.006 (Tables II and III) and it did not retain significance after correction for multiple testing. In the erosive hand OA group ($n = 197$) none of the two SNPs rs1805013 and rs1805015 showed association (Tables II and III).

When considering the distribution of SNP rs1805013 according to the radiological damage scores, no significant differences were observed among patients homozygous for the major allele, heterozygous and homozygous for the minor allele (Table IV).

CMC1 OA is a particular subtype of hand OA which develops at the base of the thumb. In this subgroup ($n = 166$), none of the *IL-4R* SNPs approached the significance threshold (Table II).

None of the *IL-13* SNPs analyzed showed association with hand OA.

Some of the analyzed SNP within the *IL-4* gene showed significant association with hand OA only when considering subgroups of patients. With respect to the CMC1 OA group, two SNPs in *IL-4* (rs2243250 and rs2243274) showed association with a *P*-value of 0.027 and 0.018 respectively (Table II). These associations were attributable to an increased frequency in the probands of the minor allele at these SNPs. These two SNPs rs2243250 and rs2243274 approached the significativity threshold also when considering only patients with a non-erosive phenotype with a *P*-value of 0.048 and 0.023, respectively (Table II). None of these SNPs remains significantly associated after correction for multiple testing.

The pair-wise LD coefficient r^2 was calculated for all SNPs by using data from all cohorts genotyped (cases, cases and controls combined). With respect to chromosome 16, moderate LD could be detected (Fig. 1).

With respect to chromosome 5, this revealed strong LD between rs1881457 and rs1800925 with an r^2 of 0.86. These SNPs are both in the promoter of *IL-13*, in position −1512 and −1112, respectively. In addition, strong LD is present among all the SNPs of *IL-4*. As expected, there is no LD between *IL-13* and *IL-4* genes (Fig. 1). Analyses for haplotypes did not reveal any statistically significant signals.

Discussion

We investigated the association between polymorphisms in a series of patients with hand OA in three candidate genes of the *IL-4*/*IL-4R* system: *IL-4R*, *IL-4*, *IL-13*.

We found a positive association with two SNPs in the gene encoding *IL-4Rα* chain (rs1805013 and rs1805015) in the whole sample of OA patients. Remarkably, this association sharply increases when considering the patients with non-erosive hand OA and the association for rs1805013 remains significant also after correction for multiple testing. From our results, the non-erosive hand OA phenotype is associated with the T allele of SNP rs1805013 whereas this association disappeared when considering the more severe erosive phenotype. This result suggests that other

Table II
Association analysis of the *IL-4R*, *IL-4* and *IL-13* SNPs for the whole sample and the subgroups analyzed

Whole sample (cases $n = 403$; controls $n = 322$)					Erosive (cases $n = 197$; controls $n = 322$)			Non-erosive (cases $n = 159$; controls $n = 322$)			No hip OA (cases $n = 354$; controls $n = 322$)			CMC1 OA (cases $n = 166$; controls $n = 322$)		
Gene	NCBI SNP	Assoc allele	Chi-square	*P*-value	Assoc allele	Chi-square	*P*-value	Assoc allele	Chi-square	*P*-value	Assoc allele	Chi-square	*P*-value	Assoc allele	Chi-square	*P*-value
Chromosome 16																
IL-4R	rs2057768	T	0.023	ns*	C	1.114	ns*	T	0.391	ns*	T	0.024	ns*	T	0.859	ns*
IL-4R	rs2107356	T	1.134	ns*	T	2.572	ns*	T	0.186	ns*	T	0.897	ns*	T	0.399	ns*
IL-4R	rs1805010	A	0.12	ns*	A	0.742	ns*	A	0.002	ns*	A	0.196	ns*	G	0.560	ns*
IL-4R	rs2234895	T	0.191	ns*	C	0.006	ns*	T	0.582	ns*	T	0.064	ns*	T	0.565	ns
IL-4R	rs1805011	C	0.032	ns*	C	0.015	ns*	A	0.042	ns*	C	0.009	ns*	A	0.829	ns
IL-4R	rs1805013	T	6.366	0.0116‡	T	3.708	0.054	T	9.758	0.002‡	T	5.625	0.017‡	T	3.072	ns*
IL-4R	rs1805015	C	4.683	0.0305‡	C	1.542	ns*	C	7.563	0.006‡	C	3.613	0.057‡	C	2.309	ns*
IL-4R	rs1801275	A	1.238	ns*	G	0.228	ns*	G	2.755	ns*	G	0.697	ns*	G	0.037	ns*
IL-4R	rs1805016	G	0.545	ns*	T	0.003	ns*	G	3.457	ns*	G	0.343	ns*	G	0.393	ns*
Chromosome 5																
IL-13	rs1881457	C	0.036	ns*	C	4.127	0.042‡	T	4.314	0.037‡	C	0.269	ns*	T	0.855	ns*
IL-13	rs1800925	C	0.101	ns*	C	2.869	ns*	T	2.441	ns*	C	0.442	ns*	T	0.333	ns*
IL-13	rs20541	G	0.126	ns*	A	1.509	ns*	G	3.625	ns*	A	0.119	ns*	G	1.113	ns*
IL-13		C	0.139	ns*	C	0.938	ns*	C	0.008	ns*	C	0.079	ns*	C	0.280	ns*
IL-4	rs2243250	C	2.856	ns*	C	1.104	ns*	C	3.878	0.048‡	C	1.670	ns*	C	4.876	0.027‡
IL-4	rs2070874	T	1.981	ns*	T	1.123	ns*	T	2.304	ns*	T	1.266	ns*	T	3.301	ns*
IL-4	rs2227284	C	1.829	ns*	C	0.580	ns*	C	2.712	ns*	C	1.077	ns*	C	1.929	ns*
IL-4	rs2243266	G	1.125	ns*	G	0.312	ns*	G	2.079	ns*	G	0.556	ns*	G	3.577	ns*
IL-4	rs2243274	G	3.468	ns*	G	1.404	ns*	G	5.195	0.023‡	G	2.441	ns*	G	5.563	0.018‡

* ns, no significant difference among group.
‡ No significance after correction for multiple testing.

Table III
Genotype and allele frequencies of the polymorphisms rs1805013 and rs1805015 in the *IL-4R* gene between cases and controls

	Whole sample *n* (%)		No hip *n* (%)		Non-erosive *n* (%)	
	Controls ($n = 322$)	Cases ($n = 403$)	Controls ($n = 322$)	Cases ($n = 354$)	Controls ($n = 322$)	Cases ($n = 159$)
rs1805013						
Genotype						
CC	290 (90.1)	348 (86.4)	290 (90.1)	306 (86.4)	290 (90.1)	133 (83.6)
CT	27 (8.4)	37 (9.2)	27 (8.4)	32 (9)	27 (8.4)	18 (11.3)
TT	2 (0.6)	14 (3.5)	2 (0.6)	12 (3.4)	2 (0.6)	7 (4.4)
Allele						
C	607 (94.3)	733 (90.9)	607 (94.3)	644 (91)	607 (94.3)	284 (89.3)
T	31(4.8)	65 (8.1)	31(4.8)	56 (7.9)	31(4.8)	32(10.1)
rs1805015						
Genotype						
TT	230 (71.4)	256 (63.5)	230 (71.4)	228 (64.4)	230 (71.4)	95 (59.7)
TC	76 (23.6)	126 (31.3)	76 (23.6)	108 (30.5)	76 (23.6)	55 (34.6)
CC	11 (3.4)	15 (3.7)	11 (3.4)	13 (3.7)	11 (3.4)	8 (5)
Allele						
T	536 (83.2)	638 (79.2)	536 (83.2)	564 (79.7)	536 (83.2)	245 (77)
C	98 (15.2)	156 (19.4)	98 (15.2)	134 (18.9)	98 (15.2)	71 (22.3)

susceptibility genetic factors might probably be associated to the erosive phenotype. Anyway, the significance observed in the whole sample is not completely due to the non-erosive hand OA patients, as the distribution of the genotype at these SNPs did not significantly differ when comparing the erosive and non-erosive subgroups. In addition, when considering SNP rs1805013, we did not find any correlation between the T allele carrier status and radiological damage evaluated by two different score systems. Consequently, we suggest that SNP rs1805013 is not associated to disease severity but only to disease susceptibility.

The associated T allele of SNP rs1805013 results in an amino acid substitution of serine 436 with leucine in the codon of IL-4R and consequently in the substitution of a polar hydrophilic residue with an aliphatic hydrophobic one, a change that may have effects on protein functions. Functional studies for the S436L substitution have not been reported yet and they might clarify its role in chondrocytes mechanotransduction.

Polymorphisms in the *IL-4R* gene had been found associated with hip OA in a study published by Forster *et al.* in 2004[19]. Forster *et al.* tested nine SNPs within the *IL-4R* gene and found a significant association for two of them (S411L, here S436L, rs1805013, and S727A, here S752A, rs1805016) with hip OA. This genetic association was weak and did not incorporate a correction for multiple comparisons. In our study, we investigate all the nine SNPs already studied by Forster *et al.* in order to verify whether their results could be supported in our sample of hand OA. Our study broadly supports *IL-4R* gene polymorphisms association with OA. It is noteworthy that in the present study the association we found cannot retain its significance after multiple testing correction. In the hip OA study, the strongest association found was with SNP rs1805016 (S752A), but no association with this SNP, even at subgroup analysis level and also before multiple testing correction, was found in our study, suggesting that different loci inside the IL-4/IL-4R system may be involved in hip and hand OA susceptibility.

Interestingly, the analyzed markers surrounding rs1805013 did not show a significantly high LD with this SNP, suggesting that other untested polymorphisms in this region could be possibly associated to hand OA. Instead, the strong LD found in the other two genes suggests that no other associated variants should be present in our sample population. It is worth noting that our results are specific to Caucasian populations and the effects in other populations are unknown.

Table IV
Radiographic score in OA patients dividing according to major and minor allele carriage of *IL-4R* SNP rs1805013. Radiographic score values are expressed as median (25th–75th percentiles)

Radiological score	Major allele ($n = 351$)	Minor allele (heterozigote) ($n = 38$)	Minor allele (homozigote) ($n = 14$)	*P*-value
Kellgren–Lawrence	26.0 (17.0–40.0)	24.0 (13.0–44.0)	26.0 (17.0–34.0)	ns*
Kallman, global score	86.0 (68.0–103.0)	84.0 (72.0–112.0)	86.5 (73.0–96.5)	ns*
Osteophyte score	21.0 (14.0–28.0)	21.0 (14.0–34.0)	22.0 (11.0–27.0)	ns*
Joint space narrowing score	19.0 (14.0–27.0)	21.0 (13.0–33.0)	21.0 (17.0–27.0)	ns*

* ns, no significant difference among groups.

Our study only partially supports the findings by Forster *et al.* using a different OA outcome. A recent review failed to find genetic association between *IL-4R* and hip OA[25]. Various factors may have contributed to the discordant results among studies, including differences in the studied phenotypes and the genetic environment and, more specifically, failure to take into account factors that modulate the effect of a gene on the risk of OA. One explanation for the divergent findings may also be that, since different joints are under dissimilar loading, they may also have different causes for development of OA. For instance, the hip and knee are weight-bearing joints whereas hand joints are under very different usage and load.

Polymorphisms in *IL-4* and *IL-13* genes had been reported in patients with immune mediated disorders[26], but so far no association study has been conducted in patients with OA.

For *IL-4* gene polymorphisms, no association was found in our total population. Conversely two SNPs were associated with the CMC1 OA subset. Even though this association does not remain statistically significant after correction for multiple testing, our results show for the first time a possible association between CMC1 OA and polymorphisms in the genes of the IL-4/IL-4R system. No association was observed with the four *IL-13* SNPs.

We are aware of the small sample size and of the weakness of the association we found for *IL-4* polymorphisms and CMC1 OA. The present study may encourage to further study the relationship between this particular phenotype of hand OA and genes of the IL-4/IL-4R system in larger case series.

For this analysis we relied on strict criteria to identify very clear cut subpopulation groups, thus excluding from subgroup analyses the patients with intermediate radiological features. We are aware of the strong limitations and caveats inherent to subgroup analysis, therefore confirmation studies in independent populations are warranted on erosive and non-erosive hand OA subsets, as well as for CMC1 subset.

An issue that we do not have the opportunity to consider is the gender effect, which could have not been evaluated given the small number of male subjects in our sample. Further studies with a larger series of patients will allow the evaluation of the gender effect on this genetic association.

Association studies of several genes have attempted to elucidate the genes in which genetic variation contributes to hand OA susceptibility. There are many potential candidate genes for hand OA. So far only three genes (*A2BP1*[26], *ENPP1*[27] and *HFE*[28]) showed convincing association signals or have been reported to be associated with hand OA in at least two independent samples. Other candidate genes such as and the interleukin-1 genes (*IL-1A*, *IL-1B* and *IL-1RN*[29]), the gene for the major collagen in cartilage (*COL2A1*[30]), interleukin-6 (*IL-6*[31]), insulin-like growth factor I gene (*IGF-1*[32]), vitamin D receptor (*VDR*[33]) and the disintegrin and metalloproteinase domain 12 (*ADAM12*[34]) to date have been investigated in one or two studies and have showed suggestive genetic association with weak association signals rarely replicated in independent studies. Similarly to these studies, our results suggest a weak genetic association. On the other hand, our results rely on a small sample whereas some of these association studies have been performed on larger samples.

The present study is an attempt to replicate genetic association for genes IL-4/IL-4R system. Our results show an association between a genetic variant in the coding region of *IL-4R* gene and hand OA in Caucasian population. Although our study, in keeping with previous data on hip OA[19], confirms the suggestion that IL-4/IL-4R system plays a role in OA pathogenesis, this genetic association requires confirmation in well powered studies.

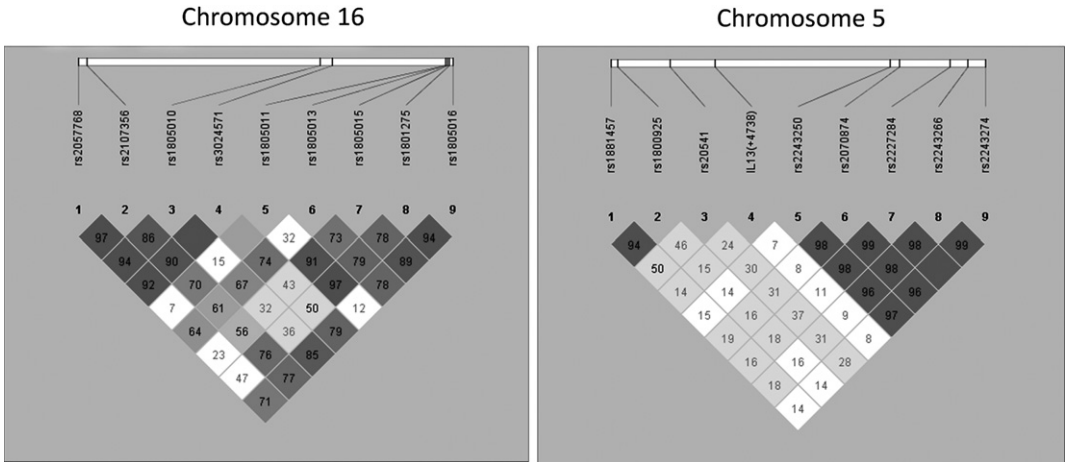

Fig. 1. Haploview output showing LD relationships between the 18 SNPs of IL-4R (chromosome 16), IL-4 and IL-13 (chromosome 5). The matrix indicates the D′ value between each pair of SNPs — darker colours indicate higher values.

Conflict of interest

The authors have no competing interest to declare.

Acknowledgements

This work was supported by grants from the Rizzoli Orthopaedic Institute, MIUR, University of Bologna and " Monte di Bologna e Ravenna" Foundation. MV was supported by a PhD fellowship from the University of Turin.

Sponsors had no involvement in any step of the study.

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